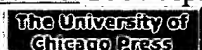


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Further characterization of *Escherichia coli* brain microvascular endothelial cell invasion gene *ibeA* by deletion, complementation, and protein expression.

Huang SH, Wan ZS, Chen YH, Jong AY, Kim KS.

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The *ibeA* gene (*ibe10*) previously identified by *TnphoA* mutagenesis is part of a 50-kDa full-length open-reading frame (ORF) encoded by a 1.37-kb DNA fragment. An isogenic in-frame deletion mutant of *ibeA* (ZD1) was constructed by chromosomal gene replacement with a suicide plasmid pCVD442 carrying a 2.1-kb DNA fragment with an *ibeA* deletion. Similar to the previously described *TnphoA* insertion mutant of *ibeA*, the isogenic *ibeA* deletion mutant ZD1 was significantly less invasive in human brain microvascular endothelial cells (BMECs) than the parent strain. The mutant ZD1 was fully complemented by the *ibeA* ORF. The *ibeA* gene was subcloned into pET28a(+) and was expressed as a recombinant protein with an N-terminal histidine tag. The recombinant IbeA protein had much greater activity (50 times) in blocking the invasion of BMECs by *Escherichia coli* K1 than did the partial protein fragment, which provides further evidence that *ibeA* is an important determinant for *E. coli* K1 invasion of BMECs.

PMID: 11237832 [PubMed - indexed for MEDLINE]



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Analysis of the protein sequence deduced from orf29, using the SignalP program (<http://www.cbs.dtu.dk/services/SignalP/>) (Nielsen, H., et al., 1997), shows that this protein has a C-terminal signal sequence with a predicted cleavage site between positions 30 and 31 (QSA/QA). It may be predicted that this protein is extracellular. It might, as a glycosylhydrolase, have a role in the reactivation of spiramycin inactivated by glycosylation by the glycosyltransferases GimA and/or GimB (Gourmelen et al, 1998).